US PAT NO: 5,705,345 [IMAGE AVAILABLE] L8: 1 of 2
DATE ISSUED: Jan. 6, 1998
TITLE: Methods and kits for preparing nucleic acids using cyclodextrin
INVENTOR: Arne Lundin, Dalaro, Sweden
John George Anson, Cardiff, Wales
Michael Kenneth Kenrick, Cardiff, Wales
ASSIGNEE: Amersham International plc, Buckinghamshire, United Kingdom (foreign corp.)
APPL-NO: 08/645,688
DATE FILED: May 14, 1996
ART-UNIT: 187
PRIM-EXMR: Kenneth R. Horlick
LEGAL-REP: Wenderoth, Lind & Ponack

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US PAT NO: 5,705,345 [IMAGE AVAILABLE] L8: 1 of 2
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US PAT NO: 5,558,986 [IMAGE AVAILABLE] L8: 2 of 2
DATE ISSUED: Sep. 24, 1996
TITLE: Method and kit for ATP assays using cyclodextrin
INVENTOR: Arne Lundin, Dalaro, Sweden
ASSIGNEE: Merck Patent GmbH, Darmstadt, Federal Republic of Germany (foreign corp.)
APPL-NO: 08/347,228
DATE FILED: Nov. 23, 1994
ART-UNIT: 187
PRIM-EXMR: Kenneth R. Horlick
LEGAL-REP: Wenderoth, Lind & Ponack

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US PAT NO: 5,705,345 [IMAGE AVAILABLE] L8: 1 of 2

SUMMARY:

BSUM(6)

The . . . or identification. The purification of genomic DNA from cells or tissue for subsequent use in gene analysis experiments conventionally involves **cell lysis** to release all **cellular** components, followed by selective digestion of proteins and RNA with specific degradative enzymes. After separation from proteinaceous material and other. . . where functionally active genomic DNA can be prepared without specific removal of contaminating protein, for example by ethanol precipitation of **cell lysates** (H. Xu, A. M. Jevnikar and E. Rubin-Kelly, Nucleic Acids Research 18, 4943). The critical contaminant therefore appears to be. . .

SUMMARY:

BSUM(37)

A . . . of potential extractants was selected among various surfactants known from preliminary experiments to rapidly inactivate firefly luciferase. The extractants included **dodecyl trimethyl ammonium bromide** (DTAB; Sigma Chemical Co: D8638), cetyl pyridinium chloride (CPC; Sigma Chemical Co; C9002), benzalkonium chloride (BAC; ACO Lakemedel AB; 10%. . .

SUMMARY:

BSUM(87)

In this experiment, the use of cyclodextrins was investigated for detergent neutralisation after **cell lysis**. HeLa **cells** (10.sup.7) in 0.5 ml PBS (Sigma) were lysed by the addition of 1 ml of **Cell Lysis** Buffer (100 mM Tris, pH 8; 1 mM EDTA; 1% SDS; 0.4 mg/ml RNase A; 40 U/ml RNase T1). The. . .

SUMMARY:

BSUM(93)

The PCR and restriction digest experiments indicate that .alpha.CD is effective for SDS neutralisation in crude **cell lysates**, and that DNA present in these lysates is functionally active.

DETDESC:

DETD(4)

Red blood **cells** were **lysed** with the following buffer to produce nuclei at room temperature for 5-10 min.:

CLAIMS:

CLMS(1)

We . . .

a method of preparing nucleic acids comprising obtaining a sample containing cells and treating said sample with an extractant to **lyse** said **cells** and release nucleic acids, the improvement which comprises adding a cyclodextrin to the treated sample to neutralize the extractant.

US PAT NO: 5,558,986 [IMAGE AVAILABLE] L8: 2 of 2

SUMMARY:

BSUM(7)

The . . . or identification. The purification of genomic DNA from

cells or tissue for subsequent use in gene analysis experiments conventionally involves **cell lysis** to release all **cellular** components, followed by selective digestion of proteins and RNA with specific degradative enzymes. After separation from proteinaceous material and other. . . where functionally active genomic DNA can be prepared without specific removal of contaminating protein, for example by ethanol precipitation of **cell lysates** (H. Xu, A.M. Jevnikar and E. Rubin-Kelly, Nucleic Acids Research 18, 4943). The critical contaminant therefore appears to be the. . .

DETDESC:

DETD(3)

A . . . of potential extractants was selected among various surfactants known from preliminary experiments to rapidly inactivate firefly luciferase. The extractants included **dodecyl trimethyl ammonium bromide** (DTAB; Sigma Chemical Co; D8638), cetyl pyridinium chloride (CPC; Sigma Chemical Co; C9002), benzalkonium chloride (BAC; ACO Lakemedel AB; 10%. . .

DETDESC:

DETD(53)

In this experiment, the use of cyclodextrins was investigated for detergent neutralisation after **cell lysis**. HeLa **cells** (10.sup.7) in 0.5 ml PBS (Sigma) were lysed by the addition of 1 ml of **Cell Lysis** Buffer (100 mM Tri s, pH 8; 1 mM EDTA; 1% SDS; 0.4 mg/ml RNase A; 40 U/ml RNase T1).. . .

DETDESC:

DETD(60)

The PCR and restriction digest experiments indicate that .alpha.CD is effective for SDS neutralisation in crude **cell lysates**, and that DNA present in these lysates is functionally active.

1. 5,932,779, Aug. 3, 1999, Screening methods for compounds useful in the regulation of body weight; Frank Lee, et al., 800/9, 18 [IMAGE AVAILABLE]

2. 5,922,557, Jul. 13, 1999, System for stably expressing a high-affinity camp phosphodiesterase and use thereof; Douglas J. Pon, 435/21, 4, 7.6, 19 [IMAGE AVAILABLE]

3. 5,880,268, Mar. 9, 1999, Modulators of the interaction between ICAM-R and .alpha..sub.d /CD18; W. Michael Gallatin, et al., 530/387.3, 387.9, 388.1, 388.22 [IMAGE AVAILABLE]

4. 5,871,712, Feb. 16, 1999, Methods for detecting calpain activation and identifying calpain inhibitors; Robert Siman, 424/9.1; 435/7.1, 7.21 [IMAGE AVAILABLE]

5. 5,869,262, Feb. 9, 1999, Method for monitoring an inflammatory disease state by detecting circulating ICAM-R; W. Michael Gallatin, et al., 435/7.1, 7.92, 7.94, 7.95; 436/811 [IMAGE AVAILABLE]

6. 5,837,822, Nov. 17, 1998, Humanized antibodies specific for ICAM related protein; W. Michael Gallatin, et al., 530/387.3, 388.1, 388.22 [IMAGE AVAILABLE]

7. 5,837,478, Nov. 17, 1998, Method of identifying modulators of binding between and VCAM-1; W. Michael Gallatin, et al., 435/7.24, 7.1, 7.2, 7.21, 7.8 [IMAGE AVAILABLE]

8. 5,831,029, Nov. 3, 1998, Human .beta.2 integrin .alpha. subunit; W. Michael Gallatin, et al., 530/387.2; 435/331, 334, 346; 530/387.9, 388.1, 388.22, 388.7, 389.6 [IMAGE AVAILABLE]

9. 5,817,515, Oct. 6, 1998, Human B2 integrin alpha subunit antibodies; W. Michael Gallatin, et al., 435/343.2, 70.21, 325, 326, 332, 334, 343, 343.1, 346; 530/387.1, 387.9, 388.1, 388.2, 388.22, 388.7, 388.73, 388.75 [IMAGE AVAILABLE]

10. 5,811,517, Sep. 22, 1998, ICAM-related protein variants; W. Michael Gallatin, et al., 530/350; 435/69.1, 69.7, 252.3, 320.1, 325; 536/23.1, 23.4 [IMAGE AVAILABLE]

11. 5,773,218, Jun. 30, 1998, Method to identify compounds which modulate ICAM-related protein interactions; W. Michael Gallatin, et al., 435/6 [IMAGE AVAILABLE]

12. 5,728,533, Mar. 17, 1998, Human .beta..sub.2 integrin .alpha.subunit; W. Michael Gallatin, et al., 435/7.1, 7.8; 530/350, 380 [IMAGE AVAILABLE]

13. 5,536,639, Jul. 16, 1996, Methods for detecting calpain activation by detection of calpain activated spectrin breakdown products; Robert Siman, et al., 435/7.1, 7.9, 7.92 [IMAGE AVAILABLE]

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US PAT NO: 5,932,779 [IMAGE AVAILABLE] L12: 1 of 13

DETDESC:

DETD(25)

To determine intracellular cAMP concentrations, a **scintillation proximity assay** (SPA) may be utilized (SPA kit is provided by Amersham Life Sciences, Illinois). The assay utilizes .sup.125 I label cAMP,. . .

DETDESC:

DETD(67)

The . . . anchored in the cell membrane. Purification or enrichment of the MC4-R from such expression systems can be accomplished using appropriate **detergents** and lipid micelles and methods well known to those skilled in the art. However, such engineered host cells themselves may. . .

DETDESC:

DETD(69)

In . . . as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from **lysed cells** by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. The PGEX vectors are designed to. . .

US PAT NO: 5,932,779 [IMAGE AVAILABLE] L12: 1 of 13

ABSTRACT:
The present invention relates to drug screening assays, and diagnostic and therapeutic methods for the treatment of body weight disorders, such as obesity, anorexia and cachexia, utilizing the melanocortin 4-receptor (MC4-R) as the target for intervention. The invention also relates to compounds that modulate the activity or expression of the MC4-R, and the use of such compounds in the treatment of body weight disorders.

DETDESC:

DETD(25)

To determine intracellular cAMP concentrations, a **scintillation proximity assay** (SPA) may be utilized (SPA kit is provided by Amersham Life Sciences, Illinois). The assay utilizes .sup.125 I label cAMP,. . .

DETDESC:

DETD(67)

The . . . anchored in the cell membrane. Purification or enrichment of the MC4-R from such expression systems can be accomplished using appropriate **detergents** and lipid micelles and methods well known to those skilled in the art. However, such engineered host cells themselves may. . .

DETDESC:

DETD(69)

In . . . as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from **lysed cells** by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. The PGEX vectors are designed to. . .

US PAT NO: 5,837,478 [IMAGE AVAILABLE] L12: 7 of 13

ABSTRACT:
Methods to identify modulators of .alpha..sub.d binding to VCAM-1 are disclosed.

SUMMARY:

BSUM(20)

Assays . . . .alpha..sub.d binding molecules are also provided, including in vitro assays such as immobilized ligand binding assays, solution binding assays, and **scintillation proximity assays**, as well as cell based assays such as di-hybrid screening assays, split hybrid screening assays, and the like. Cell based. . .

DETDESC:

DETD(143)

Screening by **Scintillation Proximity Assay** ID of Modulators of .alpha..sub.D Binding

DETDESC:

DETD(144)

Specific . . . the present invention and their binding partners (.alpha..sub.d ligand/anti-ligand pair) may be determined by a variety of means, such as **scintillation proximity assay** techniques as generally described in U.S. Pat. No. 4,271,139, Hart and Greenwald, Mol. Inmunol. 12:265-267 (1979), and Hart and Greenwald,. . .

DETDESC:

DETD(146)

The soluble recombinant .alpha..sub.d /CD18 leucine zipper construct (see Example 14) is used in a **scintillation proximity assay** to screen for modulators of CAM binding by the following method. The recombinant integrin is immobilized with a nonblocking anti-.alpha.. . .

DETDESC:

DETD(150)

As an alternative to **scintillation proximity assays**, .alpha..sub.d binding partners and inhibitors of the same can be identified using ELISA-like assays as described below.

DETDESC:

DETD(219)

9. . . . boost two weeks after the initial immunization. Immunization by this technique prevents possible changes in protein conformation often associated with **detergent lysis** of **cells**. Additional mice are immunized with recombinant protein, also resin-bound, but were not initially immunized with protein purified from **cell lysate**.

DETDESC:

DETD(221)

10. As another alternative, monoclonal antibodies are generated as follows. Affinity purified .alpha..sub.d /CD18 heterodimeric protein from **detergent** lysates of stably transfected CHO cells is used with 50 .mu.g/ml muramyl dipeptidase to immunize Balb/c mice as described above.. . .

DETDESC:

DETD(223)

12. As another alternative, CD18 complexes from **detergent** lysates of PMA stimulated HL60 cells are enriched by preclearance as described above. Other .beta.32 integrins are cleared on the. . .

DETDESC:

DETD(266)

In . . . of the results with HL60 cells, granulocytes were isolated from peripheral blood by ficoll/hypaque gradient centrifugation and subsequent red blood **cells lysis**. All preparations were found to be >90% PMNs by visualization of nuclear morphology in acetic acid. Separate populations were stimulated. . . similar than that observed on HL60 cells. The antibody 169B was used subsequently to precipitate a heterodimeric molecule from a **detergent** lysate of biotinylated PMNs with subunit sizes of approximately 150 and 95 kD appropriate t .alpha..sub.d and CD18, respectively.

DETDESC:

DETD(381)

Following . . . at 4.degree. C. and the supernatant was collected. The pelleted beads were washed sequentially with a series of 1 ml **detergent** washes as follows: buffer #1 containing 10 mM Tris, 400 mM NaCl, 1.0% Triton X-100, pH 8.0; buffer #2 containing. . .

DETDESC:

DETD(385)

A CNBr-Sepharose affinity column with conjugated 199M monoclonal antibody was used to affinity purify rat .alpha..sub.d from spleen **cell lysates**. Briefly, approximately 1.3.times.10.sup.10 rat spleen **cells** were **lysed** in buffer consisting of 150 mM NaCl, 10 mM PMSF, 10 mM Tris, 1% Triton X-100, pH 8.0. Cells in. . .

US PAT NO: 5,766,916 [IMAGE AVAILABLE] L35: 1 of 3

ABSTRACT:
The protease necessary for polyprotein processing in Hepatitis G virus (HGV) is identified, cloned, and expressed. Proteases, truncated protease, and altered proteases are disclosed which are useful for cleavage of specific polypeptides, and for assay and design of antiviral agents specific for HGV.

DETDESC:

DETD(53)

HGV . . . affinity chromatography. The antigens can then be screened rapidly against a large number of suspected HGV hepatitis sera using alternative **immunoassays**, such as, ELISAs or Protein Blot Assays (Western blots) employing the isolated antigen peptide. The antigen polypeptides fusion can be. . .

DETDESC:

DETD(54)

The purified antigen polypeptide or fusion polypeptide containing the antigen of interest, is attached to a solid support, for example, a **multi-well** polystyrene plate. Sera to be tested are diluted and added to the wells. After a period of time sufficient for. . .

DETDESC:

DETD(60)

In a second diagnostic configuration, known as a **homogeneous** assay, antibody binding to a solid support produces some change in the reaction medium which can be directly detected in the medium. Known general types of **homogeneous** assays proposed heretofore include (a) spin-labelled reporters, where antibody binding to the antigen is detected by a change in reported. . . encapsulated reporter. The adaptation of these methods to the protein antigen of the present invention follows conventional methods for preparing **homogeneous** assay reagents.

DETDESC:

DETD(61)

In . . . to the solid support, as in the first method, or may involve observing the effect of antibody binding on a **homogeneous** assay reagent, as in the second method.

DETDESC:

DETD(132)

BS-C-1 . . . Cysteine-free medium supplemented with .sup.3 H-Valine (200 uci/ml), .sup.35 S- Methionine, and/or .sup.35 S-Cysteine (50 uci/ml) for 2 hr. The **cells** are harvested and **lysed** according to known techniques.

DETDESC:

DETD(133)

The **cell lysates** are incubated with protein-A Sepharose beads preincubated (1 hr.) with rabbit polyclonal anti-HGV antiserum, described above. The samples are incubated for 4 hr. at 4 degrees C. The samples are then washed with buffers containing mild **detergent** known to those of skill in the art. The beads are collected by centrifugation and denatured in protein denaturation buffer. . .

US PAT NO: 5,510,106 [IMAGE AVAILABLE] L35: 2 of 3

ABSTRACT:
Compositions derived from a novel viral isolate designated feline immunodeficiency virus (FIV) include the whole virus, proteins, polypeptides and, polynucleotide sequences derived from the virus; and antibodies to antigenic sites on the virus. These compositions are useful in a variety of techniques for the detection of and vaccination against FIV. Detection methods disclosed include **immunoassays** for both the virus and antibodies to the virus, and the use of polynucleotide probes to detect the viral genome. Vaccines include both wholly and partially inactivated viruses inactivated cell lines expressing FIV antigens, and subunit vaccines. Whole, live virus is also useful as a model system for predicting the behavior of human immunodeficiency virus (HIV).

ABSTRACT:
Compositions . . . compositions are useful in a variety of techniques for the detection of and vaccination against FIV. Detection methods disclosed include **immunoassays** for both the virus and antibodies to the virus, and the use of polynucleotide probes to detect the viral genome.. . .

DETDESC:

DETD(7)

Conveniently, FIV strains may be identified by Western blot analysis where purified virus is disrupted with a suitable **detergent**, e.g., sodium dodecyl sulfate, and separated on a slab gel by electrophoresis. The separated polypeptide bands are transferred from the. . .

DETDESC:

DETD(9)

FIV . . . approximately 7.8. FIV bands at a density of about 1.15 gcm.sup.3 in a continuous sucrose gradient. Western blotting of FIV-infected **cell lysate** yields major bands at approximately 22 to 28 kD, usually about 26 kD; 50 to 60 kD, usually about 55. . .

DETDESC:

DETD(23)

To . . . of at least 80% W/W, and more preferably, in at least about 95% W/W purity. Using conventional protein purification techniques, **homogeneous** polypeptide compositions of at least about 99% W/W purity can be obtained. For example, the proteins may be purified by. . .

DETDESC:

DETD(31)

Antibodies . . . plasma, serum, urine, and the like, and cell samples, such as lymphocytes. Depending on the nature of the sample, both **immunoassays** and immunohistochemical staining techniques may find use.

DETDESC:

DETD(32)

Liquid phase **immunoassays** and Western blot analysis will find use in detection of FIV in body fluids, particularly blood and urine. The use. . .

DETDESC:

DETD(40)

A variety of labels have been employed, including those which have been described above for use in **immunoassays**, particularly radionuclides. Suitable labels may be bound to the probe by a variety of techniques. Commonly employed is nick translation. . .

DETDESC:

DETD(50)

IL-2-independent . . . the starting cultures which were depleted of IL-2 did not survive the procedure. Surviving cultures were placed in individual 2-cm.sup.2 **multiwells** at a viable cell concentration of 2.times.10.sup.6 cells/ml/well. During this stage only three of starting 20 cultures survived and these. . .